

DOI: <https://dx.doi.org/10.18203/2319-2003.ijbcp20243023>

Original Research Article

Effectiveness of cocoa bean extract mouthwash against gingivitis in pregnant women in Aceh Besar

Cut Aja Nuraskin^{1*}, Reca¹, Ainun Mardiah¹, Liana Rahmayani²

¹Poltekkes Kemenkes Aceh, Aceh Besar District, Aceh, Indonesia

²Department of Prosthodontics, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia

Received: 24 July 2024

Accepted: 20 August 2024

*Correspondence:

Dr. Cut Aja Nuraskin,

Email: cutaja82@yahoo.co.id

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ABSTRACT

Background: Gingivitis and periodontitis are the most common dental support tissue problems in dentistry. Gingivitis is an inflammation of the gingiva due to the body's response to the accumulation of dental plaque bacteria in the form of *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces*, and *Spirochaeta* on the surface of the teeth. Gingivitis and nutritional status in pregnant women are related to the risk of 8.75 times the birth of low-birth-weight babies (BBLR) and premature babies. Prevention of gingivitis can be done chemically through gargling with antibacterial mouthwash. Therefore, herbal ingredients are needed that can prevent plaque and control gingivitis. This study aims to determine the effectiveness of mouthwash of cocoa bean extract against gingivitis.

Methods: This study used the post-test only control group design method. The study was conducted on pregnant women in Bayu Aceh Besar village with a total of 30 samples. The sample was divided into 3 groups that received different treatments. Group 1 was instructed to do a mouthwash with cocoa extract mouthwash while Group 2 with chlorhexidine, group 3 with mineral water. Each pregnant woman gargles for 30 seconds (20 ml) for 3 days.

Results: Analysis of the gingival index before and after using cocoa seed extract mouthwash showed that this mouthwash was more effective in reducing the gingival index with a value of $p=0.012$ ($p<0.05$).

Conclusions: Gargling with cocoa bean extract is more effective in reducing gingivitis. We recommend further studies to investigate the potential of cocoa bean extract for use in mouthwashes.

Keywords: Cocoa extract, Gingivitis, Mouthwash

INTRODUCTION

Dental and oral health is an important thing that must be considered during pregnancy. Pregnancy is an event that occurs in a woman who undergoes changes, physiological conditions followed by hormonal changes. Pregnancy can affect general health, teeth and mouth which can increase the permeability of the gingival blood vessels so that it is sensitive to local irritants such as plaque, calculus and dental caries.¹

The increase in oral diseases in pregnant women can be caused by vomiting (gagging), nausea and can increase the occurrence of dental caries, the fear of brushing teeth due

to the state of gingiva.² Gingivitis is an inflammatory reaction of the gingiva caused by the accumulation of biofilm on plaque along the gingival margin and the inflammatory host's response to bacterial products. Clinical symptoms of gingivitis include changes in color, shape, consistency, texture, and bleeding in the gums when brushing your teeth.²

Pregnancy can aggravate gingivitis, known as pregnancy gingivitis or gingivitis, from the second month of pregnancy and peak in the eighth month. Gingivitis and nutritional status in pregnant women are related to the risk of low-birth-weight babies and premature babies.³

The prevalence of dental and oral diseases in Indonesia is 25.9%, gingivitis is 96.58%.³ The prevalence of gingivitis at the age of 13 years and 14-17 years is 61.5% and decreases at the age of 35-45 years. Maintenance of dental and oral health can be done with control plaque by mechanic or chemistry.⁵ Recently, it found mouthwashes that are chemically based, this is quite dangerous for health such as the stomach, changes in taste sensation, and the formation of supragingival calculus if swallowed. Therefore, herbal mouthwashes that are antibacterial in preventing and controlling gingivitis are needed.^{6,7}

Cocoa beans play an important role in health, because they contain secondary metabolic compounds of flavonoids, tannins, alkaloids that have anti-microbial, antibacterial effects. Based on previous research, cocoa bean extract is effective in inhibiting streptococcus bacteria.^{8,9} Gargling with cocoa beans at a concentration of 12.5%, 25%, 50% has an effect on the dental plaque index, the higher the concentration given, the higher the decrease in dental plaque index.¹⁰ Based on previous research, cocoa bean extract is not toxic, and can even reduce cell histological damage in mice.¹¹ Therefore, mouthwash cocoa bean extract is suitable as a basic ingredient in mouthwash so that it can accelerate the healing of gingivitis.^{12,13}

METHODS

The type of research used is quasi-experimental research with a pretest-posttest design. This study used a post-test only control group design before and after the gargle intervention with mouthwash cocoa extract, chlorhexidine, and mineral water as well as pre-test and post-test before and after gargling with chlorhexidine, and plain water as a control group in reducing gingival index in pregnant women in Bayu Village, Aceh Besar. Research on the manufacture of simplicia and cocoa extract and phytochemical tests were carried out in the laboratory of the Aceh Ministry of Health. The research on the examination of the Gingiva index was carried out on pregnant women in Bayu Aceh Besar Village. The research was conducted from March 2024 to July 2024. The number of samples in this study was 30 pregnant women, with criteria; Do not have heart abnormalities, other common diseases, and are not allergic to mouthwashes and anti-biotics. Pregnant women, have index teeth 16, 11, 26, 36, 31, 46. Ethical approval for this study was obtained from the research ethical committee of Health Polytechnic of Health Ministry of Aceh, Indonesia (Protocol No: DP.04.03/12.7/049/2024).

Procedure

Preparation of the tools and materials used, among others; Diagnostic tools, glass dappen, masks, gloves, glass, nierbeken, beken glass, measuring cups, stirring spoons, digital scales, filter paper, mouthwash bottles. Gingiva index value form, and informed consent. The ingredients used are cocoa bean extract, menthol, saccharin, sodium benzoate, and glycerin.

The process of making mouthwash

Preparation of ingredients as much as 3 kg of wet cocoa is taken, washed clean, and split to separate the husk from the seeds. The seeds are then cut into small pieces and dried at room temperature without exposure to sunlight for 2 weeks. After drying, the seeds are finely ground into simplisia. Preparation of extraction by maceration with 98% ethanol solvent %, soak for 3x24 hours then filter separate the mixture and filtrate. Rotary evaporator to obtain a viscous extract. Making mouthwash, weigh all the ingredients needed, menthol 1 gram, saccharin 0.2 grams, sodium benzoate 0.1 grams, glycerin 10 ml, cocoa extract 1 gram. Heat 100 ml of sterile aquades at 90°C for 15 minutes and then let it sit for a few minutes. Heat 20 ml of sterile aquades, remove and add 0.2 grams of saccharin, 1 gram of menthol, 10 ml of glycerin, 0.1 grams of sodium benzoate, add 1 gram of cocoa extract. Strain with filter paper and put it in a mouthwash bottle. Use after a week of storage.

Alkaloid compound test

Prepare 1 gram of cocoa extract, add 1 ml of concentrated ammonia, and 10 ml of 0.5 N hydrochloric acid. Take a layer of hydrochloric acid and divide it into three tubes. Each tube is tested for the presence of alkaloids in the following way: Addition of Mayer reagent will produce a white deposit, Addition of Dragendorff reagent will produce a reddish deposit. The addition of Wagner reagents will produce chocolate deposits. The presence of precipitate in each test indicates the presence of alkaloid compounds.

Test for steroid compounds, terpenoids, and saponins

Prepare 10 grams of cocoa extract, add distilled water, and beat vigorously. The presence of stable foam for 30 minutes indicates the presence of saponin compounds. If the saponin test is positive, the solution is hydrolyzed with HCl and tested with the Liebermann-Burchard reagent: A green or blue color indicates the presence of steroid saponin compounds. The red color indicates the presence of triterpenoid saponin compounds.

Flavonoid compound test

Prepare 10 grams of cocoa extract, add 10 ml of 80% ethanol, then add 0.5 g of magnesium metal and 0.5 M HCl. Pink or purple color indicates the presence of flavonoids.

Organoleptic test of cocoa bean extract mouthwash

Organoleptic tests are tested daily for three weeks. Observation was carried out to see, color, aroma, taste, clarity of cocoa bean extract mouthwash. Intervention Group Examination

The intervention group was divided into 3 groups, each group consisted of 10 pregnant women. On the first day, gingival index examination in each group before being given treatment. Furthermore, the distribution of cocoa bean extract mouthwash mouthwash for group 1, chlorhexidine mouthwash for group 2, and mineral water mouthwash for group 3. Each pregnant woman was instructed to gargle at home after brushing her teeth for 30 seconds (20 ml as recommended by mouthwash) for 3 days. On the third day, a gingival index examination was carried out after being given an intervention to gargle.

Statistical analysis

Data analysis was carried out by a dependent t-test statistical test using the SPSS application.

RESULTS

The birth of the tree

This phytochemical test aims to find out the secondary metabolite compounds contained in a plant. Phytochemical tests include tests for alkaloids, steroids, terpenoids, saponins, flavonoids and phenolics. Results of phytochemical tests of cocoa extract (Table 1). The results of the content of secondary metabolites in cocoa extract contain alkaloids, steroids, terpenoids, saponins, flavonoids and phenolics (Table 1).

Table 1: Phytochemical test of Laban leaf extract.

Metabolit sekunder	Ekstrak etanol biji kakao
Fenolik	+
Tanin	+
Flafonoid	+
Steroid	-
Terpenoid	+
Saponin	+
Alkaloid	
DD	+
Mayer	+
Wakner	+

(+) positive for secondary metabolic content, (-) negative no secondary metabolite content

Organoleptic results

The results of organoleptic tests observed during three weeks of storage showed that the length of storage did not affect the color, aroma, taste, and clarity of the mouthwash (Table 2).

General data

Data collection will be carried out from March to July 2024 in Bayu Village, Aceh Besar. Based on the results of data processing, the information is presented in the form of tables and narratives. Based on (Table 3), it can be seen that

of the 30 respondents most of whom are aged as follows (Table 3).

Table 2: Organoleptic test of cocoa bean extract mouthwash.

Formula	Observation	Minggu I	Minggu II	Week III
1%	Color	Coklat	Coklat	orange
	Aroma	-	-	menthol
	Taste	Pahit	Pahit	Sweet
	Shape	Jernih	Jernih	Jernih
	Kejernihan	Jernih	Jernih	Jernih

Table 3: Distribution of respondents based on age.

Age (years)	SUM	Percentage
17-19	7 people	23.3
20-29	11 People	36.7
30-34	12 People	40
SUM	30	100

Gingivitis index test results

Based on table 4, the frequency of Gingivitis before gargling chlorhexidine shows that the highest criterion is in the category of moderate criteria as many as 8 people (80%). Meanwhile, after gargling chlorhexidine, the highest criteria were divided equally between good and moderate balanced criteria, as many as 5 people (50%) each. Gumvitis Index before and after gargling cocoa bean extract (Table 4).

Table 4: Index Gingiva before and after gargling with chlorhexidine in pregnant women in Aceh Besar.

No.	Criterion	Before		After	
		F	%	F	%
1	Good	2	20	5	50
2	Keep	8	80	5	50
3	Bad	0	0	0	0
Total		10	100	10	100

Table 5: Based on the index of teeth before gargling moutshwas cocoa bean extract.

No.	Criterion	Before		After	
		F	%	F	%
1	Good	2	20	3	30
2	Keep	6	60	7	70
3	Bad	2	20	0	0
Total		10	100	10	100

Based on table 5 of the Gingivitis index before gargling, cocoa bean extract had the highest gingiva index at the moderate criterion of 6 people (60%). Meanwhile, after gargling cocoa bean extract, the highest criterion was 7 people (70%) (Table 5).

Table 6: Gum index before and after mouthwash.

Gingivitis examination	Mean	SD
Exit pretest chlorixidine	11,70	3498
Nilai post-test chlorixidine	10,20	3910
Pretest value of cocoa extract	14,90	4040
Cocoa extract post-test value	12,80	3910
Mineral pretest value	13,20	5574
Post-test mineral value	13,60	4695

From table 6 above, it shows that there was a decrease in the mean value of gingivitis after the administration of chlorixidine mouthwash by 1.50 from pretest 11.70 to 10.20 at the posttest, and cocoa extract mouthwash by 2.1 from pretest 14.90 to 12.80 at the post-test (Table 6).

Normality test

Before conducting bivariate analysis, the study first conducted a normality test on the existing data using Shapiro-wilk, which is a normality test for a small sample (less than 50). The normality test with computer software (SPSS) and the results of the normality test (Table 7) can be stated that all the data in this study, both the pretest and posttest values of the gingivitis examination are normally distributed (sig. >0.05) with a significance level of 95% (Table 7).

Table 7: Normality test data of pretest and posttest mouthwash influence value.

Variable	Normality Test	Normality /No
Nilai pretest chlorixidine	0.142	Normal
Nilai post-test chlorixidine	0.374	Normal
Nilai pretest ekstrak kakao	0.397	Normal
Nilai posttest ekstrak kakao	0.555	Normal
Nilai pretest mineral	0.278	Normal
Nilai post-test mineral	0.328	Normal

Table 8: Results of the effectiveness test of chlorixidine mouthwash and cocoa extract in pregnant women.

Variable	Mean	P value
Gingivitis examination values before and after gargling chlorixidine	0.1500	0.043
Gingivitis examination value before and after gargling cocoa extract	0.2100	0.012
Gingivitis examination values before and after gargling with mineral water	0.0400	0.843

The results of the test regarding the effect of chlorixidine mouthwash and cocoa extract mouthwash on pregnant women in Aceh Besar can be seen in table 8. Table 8 shows

the significant value of chlorixidine $p=0.043$ ($p<0.05$), the significant value of cocoa extract $p=0.012$ ($p<0.05$) and the significant value of mineral water $p=0.843$ ($p>0.05$). This shows that there is an effectiveness of chlorixidine mouthwash and cocoa extract against gingivitis in pregnant women and ineffective from mineral water against gingivitis in pregnant women (Table 8).

DISCUSSION

This study uses a maceration technique with 96% ethanol solvent 3x24 hours. The use of ethanol as a solvent is based on its good ability to extract these compounds compared to other solvents such as methanol or water.² Phytochemical tests conducted show the presence of various active compounds that provide useful bioactivity potential.

The phytochemical content in cocoa beans is influenced by various internal and external factors. External factors that affect the composition of active compounds include: Sunlight: The intensity and duration of sunlight exposure can affect the synthesis of phenolic compounds and flavonoids. Plants that get optimal sunlight tend to have a higher content of antioxidant compounds.³ Rainfall: The availability of water from rainfall affects plant metabolism, which in turn can affect the production of phytochemical compounds.⁴ Soil structure: The mineral composition and texture of the soil play an important role in the absorption of nutrients by plants, which can affect the phytochemical content. Climate: Temperature and humidity also have a significant effect on the production of phytochemical compounds. Extreme climatic conditions can trigger stress in plants, which often increase the production of protective compounds such as phenolics and flavonoids.⁵

The maceration method with ethanol solvent is carried out by soaking cocoa beans that have been crushed in a solvent for a certain period of time. This process is followed by filtration and a rotary evaporator to obtain a viscous extract which is then further analyzed. Phytochemical tests are used to identify active compounds in cocoa beans. This study shows that ethanol extract from cocoa beans has great potential in pharmaceutical and nutraceutical applications thanks to its high content of bioactive compounds.

Alkaloid compound test

Dry sample as much as 1 gram plus 1 ml of ammonia, add 10 ml of 0.5N hydrochloric acid. The mixture is vigorously shaken and divided into three test tubes. The addition of reagents is as follows: Mayer reagent: Causes white precipitate. Dragendorff Reagent: Causes reddish deposits, Wagner Reagent: Causes brown deposits. Results: All three positive reactions indicate the presence of alkaloid compounds.

Test for steroid compounds, terpenoids, and saponins method: A dry sample of cocoa beans as much as 10 grams is added with aquades and shaken vigorously. The presence of foam indicates the presence of saponins. The samples

were then hydrolyzed with HCl and tested with Liebermann-Burchard reagents. The green or blue color indicates steroid saponins. The red color indicates triterpenoid saponins. Result: Foam showed the presence of saponins. The green or blue color indicates the presence of steroid saponins. The red color indicates the presence of triterpenoid saponins.

Flavonoid compound test method

A dry sample of 10 grams is added with 10 ml of 80% ethanol. Added 0.5 grams of magnesium metal and 0.5 ml HCl 0.5M. Pink or purple indicates the presence of flavonoids. Results: Pink or purple color indicates the presence of flavonoids. Conclusion of phytochemical test results: Methanol extract of Sare Aceh Aceh Besar cocoa beans contains various secondary metabolites, including: phenolics, tannins, flavonoids, terpenoids, saponins, alkaloids.

Organoleptic test, this test is carried out with the aim of finding out the feasibility of the mouthwash preparations made. In organoleptic testing, the odor, color and shape of the preparation are observed. The mouthwash preparations made have a liquid form which is a characteristic of mouthwashes in general. In terms of color, the preparation produces a brown color that matches the color of the brown fruit plant, in terms of smell, the preparation has no smell.^{6,7} Stability During Storage: Storage for three weeks did not affect color, aroma, taste, and clarity, indicating that the formulation of this mouthwash was stable over that period of time.^{8,9}

Based on Table 3, it can be seen that gingivitis before gargling chlorixidine with the most dominant criteria is moderate criteria as many as 8 people (80%) and good criteria 2 people (20%), but after gargling with chlorixidine there is a decrease in the value of gingivitis with good criteria as many as 5 people (50%) and moderate criteria as many as 5 people orang (50%).

Based on Table 4, it can be seen that gingivitis before gargling cocoa bean extract with the most dominant criteria are moderate criteria as many as 6 people (60%), good criteria as many as 2 people (20%) and bad criteria as many as 2 people (20%), but after gargling with cocoa bean extract there is a decrease in gingivitis values with moderate criteria as many as 7 people (70%) and good criteria as many as 3 people (30%).

Based on Table 5, it was shown that there was a decrease in the mean value of gingivitis after the administration of chlorixidine mouthwash by 15.0 from pretest 11.70 to 10.20 at the posttest, and cocoa bean extract by 2.1 from pretest 14.90 to 12.80 at the posttest. At the time of pre-test and post-test there was a significant difference, reviewed based on the results of the Paired T-Test Chlorixidine $p=0.043$ ($p<0.05$), and cocoa bean extract $p=0.012$ ($p<0.05$), at a 95% confidence level, thus it can be concluded that there is an effectiveness of gargling on

reducing gingivitis in pregnant women in Aceh Besar, Bayu Village.

Based on Table 6, the results of the normality test on the existing data can be seen using Shapiro-wilk, namely the normality test for a small sample (less than 50). The pretest value of chlorixidine was 0.142 and the posttest value of chlorixidine was 0.374, the pretest value of cocoa bean extract was 0.397 and the posttest value of cocoa bean extract was 0.555, the pretest value of mineral water was 0.278 and the posttest value of mineral water was 0.328, stating that the values were distributed normally ($\text{sig.}>0.05$) with a significance level of 95% which aimed to check whether the research data obtained came from a population with a normal distribution.

Based on Table 8 above, it shows that the significant value of chlorixidine $p=0.043$ ($p<0.05$), the significant value of cocoa bean extract $p=0.012$ ($p<0.05$) and the significant value of mineral water $p=0.843$ ($p>0.05$). This shows the effectiveness of chlorixidine mouthwash and cocoa bean extract against gingivitis in pregnant women. The results show that gargling with mouthwash can reduce bad bacteria in the mouth.

Based on the results of chlorixidine gargling, and cocoa bean extract in reducing the value of gingivitis, gargling with cocoa bean extract solution is more effective in reducing the value of gingivitis compared to gargling chlorixidine. Because cocoa plants contain alkaloids, flavonoids, saponins, sterpenoids, tannins, it is believed that cocoa plants can inhibit bacteria.¹⁰

Pregnant women often experience hormonal changes that can increase the risk of gingivitis. Inflammation of the gums during pregnancy can become a serious health problem if not treated properly. Therefore, finding a safe and effective method to treat gingivitis in pregnant women is very important.

Cocoa bean extract has been known to have a variety of bioactive compounds that are beneficial for oral health. Compounds such as alkaloids have antibacterial properties that can inhibit the growth of bacteria that cause gingivitis. Flavonoids are known to have anti-inflammatory properties that help reduce inflammation in the gums. Saponins act as natural cleaning agents that can remove plaque from the surface of the teeth. Sterpenoids and tannins also play a role in reducing inflammation and inhibiting bacterial growth. Chlorhexidine is known as a powerful antibacterial agent that is often used in oral care products untuk reduce plaque and gingivitis.¹¹ However, long-term use of chlorhexidine can cause side effects such as tooth discoloration and taste disorders, which can reduce pregnant women's compliance with their use.¹² Cocoa bean extract, on the other hand, is not only effective but also offers a more natural alternative and may have fewer side effects.

Some limitations that may be faced in the study of the effectiveness of cocoa bean extract mouthwash on gingivitis in pregnant women: limited sample size, with only 30 participants, the results of the study may not be generalizable to a wider population. The small sample size may also affect the reliability of the study results. Short study duration, participants' compliance in using mouthwash according to instructions may vary, which may affect the study results. Limitations of the assessment method: the method of assessing the effectiveness of mouthwash may not be completely objective and may be influenced by the subjective judgment of the researcher.

CONCLUSION

Conclusion from the results, a conclusion can be found, namely the effect of mouthwash on gingivitis in pregnant women. The average value of gingivitis before gargling chlorixidine was 1.11 with moderate criteria, while after gargling chlorixidine there was a decrease to 1.02 with mild criteria. The average value of gingivitis before gargling was 1.25 with moderate, while after gargling cocoa extract skin decreased to 1.02 with mild criteria. The results of the Paired T-test showed the difference in gingivitis values before and after gargling chlorixidine (0.1500), gargling cocoa extract (0.2100) and gargling with mineral water (-0.0400) thus the average difference between gargling cocoa extract was higher than gargling with chloroxidine and mineral water in reducing the value of gingivitis.

Recommendations

The recommendations further research is encouraged to investigate the potential of cocoa extract as a basic ingredient in mouthwashes.

Funding: Funded by DIPA Poltekkes Kemankes Aceh 2024

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Nuraskin CA, Reca, Mardiah A, Rahmayani L. Effectiveness of cocoa bean extract mouthwash against gingivitis in pregnant women in Aceh Besar. *Int J Basic Clin Pharmacol* 2024;13:776-81.